2.0 METHODS.

2.1 Source of Study Fish

All chinook salmon used in the study were of hatchery origin. Fish were obtained from Little White Salmon National Fish Hatchery, Washington (Figure 1-1). The hatchery is located about 25 mi north of Bonneville Dam. Fish were transported daily from the hatchery in a 100 gal fish transport tank mounted in a pick-up truck to the study site in lots of 100 to 400 fish. Fish were acclimated for about 24 h to the ambient water temperature of the Columbia River at the study site prior to being tagged and released. The procedure used to gradually acclimate fish to the ambient river water was in accordance with the guidelines prepared by the U.S. Fish and Wildlife Service hatcheries. The difference in the two temperatures was generally less than 4.0°C (7.2°F). Ambient water temperature dropped from 14.5°C to 12°C (58.1°F to 53.6°F) during the study. The fish transport tank was equipped with a recirculation system and supplemental oxygen supply. The transport time from the hatchery to the study site was less than three hours.

Treatment and control fish were drawn from the same group of fish assuring similar size and condition. Figure 2-1 shows the length frequency distribution of the treatment and control fish for the three treatment and control groups. The average total length was almost identical for the three groups (128 to 130 mm total length treatment and 130 to 132 mm control).

2.2 Sample Size

One of the main considerations was to obtain an estimate of survival/fish condition within a specified precision level ($\varepsilon \leq \pm 0.05$, $\alpha = 0.10$) for each test condition using a relatively small number of fish (sample size). Because the objective of the study was not to delineate statistical differences ($P < 0.05$) of specific magnitude between the two treatment conditions, sample size calculations were not made for that purpose. Undoubtedly, the sample size requirements would be higher if one desires to detect a modest difference, say about 3%, between the two test conditions with a reasonable statistical power ($1-ß = 0.80$) at $\alpha = 0.05$ (RMC *et al.* 1994). Statistical power is a function of α, treatment effect size to be detected, sample size, and variance. Statistical significance (α) and treatment effect size to be detected can be pre-specified by investigators, estimate of variance needs to be obtained empirically or from other studies. Should a need arise in the future to perform such calculations, the estimates of the present study can be used.

The sample size is a function of the recapture probability (P), passage survival () or mortality (1-), survival probability of control fish (S), and the desired precision (ε) at a given probability of significance (α). In general, sample size requirements decrease with an increase in control survival and recapture probabilities. Figure 2-2 shows an example relationship of control survival and recapture probabilities and the sample size for achieving a precision level (ε) of $\leq \pm 0.05$, $\alpha = 0.10$. In the present case, results of some previous survival investigations at hydroelectric dams on the Columbia River and the

Snake River (RMC and Skalski 1994a,b; RMC *et al.* 1994; Normandeau Associates *et al.* 1995) provided preliminary estimates on the recapture and control survival probabilities to calculate sample sizes for this study. The derivation of precision is shown in Appendix A.

At the Rocky Reach Dam on the Columbia River, recapture probabilities of treatment fish were 0.885 to 0.955 and for control 0.935 to 0.988; control survival 0.935 to 0.988; and the immediate survival was 0.94 to 0.96 (RMC and Skalski 1994a,b). It was concluded that a sample size of 250 fish each for treatment and control groups would be adequate for achieving a precision (ε) of $\leq \pm 0.05$ ($1-\alpha=0.90$) on the survival estimate. In the 1994 study at Lower Granite Dam the recapture probabilities for the treatment and control groups were 0.945 and 0.988, respectively, and the estimated immediate (1 h) passage survival () was 0.946 (RMC *et al.* 1994). In the 1995 survival research at Lower Granite Dam, the recapture probabilities for the treatment and control groups exceeded 0.96 (range of 0.964 to 0.996), with immediate (1 h) passage survival probabilities ranging from 0.946 to 0.975 (Normandeau Associates *et al.* 1995). The study also concluded that a shared control release for two treatment releases was a viable experimental protocol. This release protocol also proved viable in the recent Bonneville Dam spillway study (Normandeau Associates *et al.* 1996). Using the range of values obtained in the above studies and the variance expressions given in Appendix A, potential sample sizes were calculated prior to initiating the study (Tables 2-1 and 2-2). We assumed a control survival (S) probability of 0.95 or 0.99, recapture probabilities (P) of 0.90, 0.95, and 0.99, passage survival (τ) of 0.95, 0.90, 0.925, and 0.85. These calculations showed that a precision (ε) of $\leq \pm 0.05$ on the point estimate of survival at alpha (α)=0.10 (the confidence level specified by ACOE) for each test condition could be achieved with a paired release of 311 fish if the expected passage survival is 0.925, recapture probability is 0.90, and control survival is 0.99 (Table 2-1). With control survival probability of 0.95, recapture probability of 0.95, and expected passage survival of 0.925, a sample size of 284 fish each for treatment and control group would be needed (Table 2-2). It was further assumed that since the results of daily releases would be readily available, sample sizes could be adjusted accordingly.

2.3 Release Locations

The original ACOE study plan had envisioned three experiments for obtaining estimates of differential fish survival and condition at three different spillbays each discharging about 10,000 cfs. The three spillbays included spillbay 3 (unmodified), spillbay 4 (I-slot), and spillbay 6 (overflow weir). However, hydraulic/engineering field testing of spillbay 6 overflow weir at flows greater than 5,000 cfs indicated serious vibrations in the tainter gate and structural components. Therefore, a lower spill (4,500 cfs) through spillbay 6 was discharged to complete the fish survival experiment. The lower spill was deemed safer from an engineering viewpoint.

The treatment fish for tests at spillbays 3 and 4 were released at a pre-specified fixed location and spill of approximately 10,500 cfs. For the test at spillbay 6, the spill was 4,500 cfs. The depth and

location of the hose for the treatment fish was determined in consultation with the ACOE personnel so the fish would exit into an area of sufficient velocity (>5 ft/sec) to prevent escape upstream (Figures 1-3 and 1-6). The release hose was mounted so its terminus for both the I-slot and overflow weir was in the middle of the bay and about 6 ft below the surface (Steve Dingman, ACOE, The Dalles Dam, pers. comm.). For the unmodified bay, the release hose terminus was also mid-bay and about 20 ft below the surface. All release hoses were approximately 9 ft upstream of the tainter gate. Control fish were released downstream of spillbay 3 (Figure 2-3). The end of the control release hose was positioned approximately 15 ft above the water and oriented to discharge fish and water in the direction of the flow.

All control specimens were released from the same location; however, discharge through spillbay 3 was reduced to 4,500 cfs for the overflow weir test.

Prior to initiating the full-scale study a "shake-down" was conducted. This involved releasing 5 to 10 treatment fish through the spillbay and control sites to identify any snags in the experimental procedures and protocols, safety concerns, expected recapture rates, and where the tagged fish were expected to surface. Upon satisfactory completion of the "shake-down" the full scale study at spillbays was initiated on 27 October 1995.

In addition to the primary test conditions, the ACOE was also interested in obtaining a general idea on potential fish passage problems associated with the ice and trash sluiceway at the powerhouse (Figures 1-2 and 2-4). Consequently, 100 fish were also released through the ice and trash sluiceway, no control fish were concurrently released. Fish were released at the water surface; about 50 ft upstream from the edge of the sluiceway. Because of the limited scope of the ice and trash sluiceway study, results of this experiment are presented separately in Section 3.3.

2.4 Tag-Release Scheme

Tagging and release techniques were similar to those described for estimating direct effects of turbine or spill passage in Heisey *et al.* (1992) and RMC and Skalski (1994a,b). Fish were anesthetized in 0.5% MS-222, held in a 4 gal tub and tagged with two HI-Z tags and a miniature radio tag. Additionally, each fish was given a uniquely numbered visual implant VI tag (Northwest Marine Technology, Inc., Shaw Island, Washington), for tracking survival and condition of individual smolts held over the 48 h period. Because initial releases indicated some loss of VI tags, all succeeding specimens received a fin clip in addition to the VI tag prior to release. When fish were fully recovered from anesthesia they were individually placed into the induction system holding tub (Heisey *et al.* 1992), tags activated, and fish released. Tagging crews were rotated daily so that each team released similar numbers of treatment and control fish. Normally a tagging crew tagged and released a total of 30 to 80 treatment or control fish on each day. The treatment fish for the test conditions were released through an induction apparatus at a fixed location (Figures 1-3 and 1-6) in each spillbay at a constant spill volume. Each induction apparatus consisted of a small holding basin attached to a 4 in diameter induction hose line and was supplied with

ambient river water to ensure that fish were transported quickly within a continuous flow of water. The flexible hose was passed through a 6 in diameter metal pipe to secure it at the desired release location.

Fish were randomly selected from each day's transport. Lots of 5 to 10 treatment and control fish were alternately released throughout the day. We released 210 to 270 treatment fish for the three test conditions, along with 105 to 230 matching controls (Table 2-3). We released 270 and 271 treatment fish (6 to 7 trials of 30 to 81 fish each) for spillbays 3 and 4 tests, respectively. For the first five trials a common control of 30 to 40 fish each day was released. However, due to logistical constraints, priority of releases, and the need for moving of release structures, we had to modify control release protocol for the subsequent three days. On the sixth day we released 81 treatment fish for spillbay 4 with a matching control of 40 fish; no spillbay 3 testing occurred. On days 7 and 8, we tested spillbay 3 with treatment releases of 40 fish each day matched with control releases of 20 fish each day (Table 2-3). This release scheme proved logistically effective. It provided some economy and utilized a relatively smaller number of fish without sacrificing precision. Matched treatment and control releases for spillbay 6 were made on the subsequent three days (Table 2-3).

2.5 Fish Recapture

Shortly after release (generally two to five minutes) the tags inflated and buoyed the fish to the surface for rapid recapture by a recovery boat crew. Both treatment and control fish were retrieved from the tailwater by up to four boat crews. Recovery boat crews were notified of the radio tag frequency of each fish upon its release. To minimize crew bias, no crew was specifically assigned to retrieve either control or treatment fish (Mathur *et al.* 1996a). Only crew members trained in fish handling retrieved tagged fish.

As a precautionary measure, the ACOE secured the services of personnel from the U.S. Department of Agriculture to scare the gulls from the tailrace. Past experience had shown that hazing of gulls minimizes the potential loss of buoyed experimental fish thus maintains the use of pre-specified sample sizes. Dispersing stale bread also proves effective in attracting gulls to areas away from the fish recapture sites (Normandeau Associates *et al.* 1996). However, predation by gulls on tagged fish was not observed in the present study.

Radio signals were received on a 5-element Yagi antenna coupled to an Advanced Telemetry systems programmable scanning receiver. The radio signal transmission enabled the boat crew(s) to follow the movement of each fish after spillway passage and position the boat for quick retrieval when the balloon tag buoyed the fish to the surface. The boats maintained a safe distance downstream of the turbulent water in the spillbay (Figure 2-3). For safety reasons, spill was curtailed to recapture buoyed fish that became entrapped in turbulent areas for more than 15 min. Fish with active radio tags that failed to surface were tracked for 30 minutes and then periodically to ascertain if fish were displaying movement patterns typical of emigrating smolts, stationary signals, or that of a predator. Buoyed fish were retrieved

and placed into an on-board holding facility. The tag(s) were removed by a pin puller (modified pliers). Each fish was examined for descaling and injuries and assigned codes relative to descriptions presented in Table 2-4. Tagging and data recording personnel were notified via a two-way radio system of each fish's recovery time and condition.

Each recaptured fish was immediately examined for physical injuries. Because controlled experiments replicating and correlating each injury type/characteristics to a specific causative mechanism are lacking in the literature, a definitive classification of observed injuries in the field, particularly in the case of multiple injuries, is difficult (Eicher Associates 1987). Thus, only probable causes could be attributed to the observed fish injuries.

All fish recaptured alive were transferred in covered 5 gal pails as soon as possible to 600 gal holding pools located on a lower deck at the Oregon side of the spillway. Each day's treatment and control fish were held in the same pool for 48 h. Pools were continuously supplied with ambient river water and shielded to prevent fish escapement and potential avian predation.

2.6 Classification of Recaptured Fish

Buoyed recaptured fish and recovery of inflated tags only (those dislodged from fish) were classified as described in Normandeau Associates *et al.* (1996) to estimate the immediate (1 h) and 48 h effects of passage. Immediate status of each fish was designated alive, dead, predation, or unknown. The following criteria are used to make these designations: (1) alive--recaptured alive and remained so for 1 h; (2) alive--when radio signals from non-recaptured fish indicated movement patterns typical of emigrating juveniles; (3) dead--recaptured dead or dead within 1 h of release; (4) dead--recovery of inflated tag(s) without fish and telemetric tracking (stationary signals) or the manner in which tags surfaced not indicative of predation; (5) unknown--when nothing was recaptured and the exact status could not be ascertained from the radio signals; (6) predation--when fish were either actually observed being preyed upon, predator was buoyed to the surface, or subsequent radio telemetric tracking and/or tag indicated predation (i.e., rapid movements of tagged fish in and out of turbulent waters or sudden appearance of fully inflated dislodged tags).

Mortalities which occurred >1 h after fish were released through the induction apparatus were assigned a status delayed mortality (48 h). Fish held in pools were observed approximately at 12 h intervals. Dead fish were identified by the numbered VI tag, examined for descaling and injury, and necropsied to determine the potential cause of death. Additionally, all specimens alive at 48 h were re-anesthetized and closely examined for injury and descaling. Injury and descaling were categorized by type, extent, and area of body. This re-examination of immobilized fish minimized additional handling stress immediately upon recapture. The descaling recorded on each fish during the detailed examination provided a better estimate than that recorded upon immediate recapture. Criterion used for descaling was in accordance with that recommended by the ACOE for smolt monitoring. Injuries were recorded at the

initial recapture and later during the detailed examination at 48 h. This procedure was followed because some injuries, such as bleeding, were no longer evident at 48 h and some additional injuries were detected during the detailed examination (Normandeau Associates *et al.* 1996).

2.7 Assumptions

The following explicit assumptions were made in obtaining a valid estimate of injury/mortality rate: tagging, handling, and release do not differentially affect survival of treatment and control fish groups; treatment and control groups are equally vulnerable to recapture; and recovery crews do not differentially retrieve treatment and control groups. Statistical analyses were also performed to support the viability of these assumptions (RMC and Skalski 1994a,b).

2.8 Data Analysis

Passage survival rates of fishes are estimated using paired release-recapture methods (Ricker 1975; Burnham *et al.* 1987). Unlike earlier investigations, however, recaptures of both alive and dead fish are possible with the HI-Z tag-recapture technique (Heisey *et al.* 1992). Thus, parameters associated with both alive and dead fish can be incorporated into the construction of a statistical model. This, along with high recapture probabilities, can be used to precisely estimate passage survival rates (Mathur *et al.* 1996a). Separate survival estimates were calculated for each release scheme.

The following terms are used in the equations and likelihood functions that follow:

R_c = Number of control fish released,

R_T = Number of treatment fish released,

a_c = Number of control fish recaptured alive,

d_c = Number of control fish recaptured dead,

a_T = Number of treatment fish recaptured alive,

d_T = Number of treatment fish recaptured dead,

S = Probability fish survive from the release point of the controls to recapture,

P_A = Probability an alive fish is recaptured,

P_D = Probability a dead fish is recaptured,

= Probability a treatment fish survives to recapture point (*i.e.*, passage survival),

1- = Passage mortality.

The joint likelihood for the passage-related mortality (Skalski 1992) is as follows:

$$L(S,\ t,\ P_A,\ P_D | R_C,\ R_T,\ a_C,\ d_C,\ a_T,\ d_T) =$$

$$\binom{R_c}{a_c, d_c} (SP_A)^{a_c}((1-S)P_D)^{d_c} (1-SP_A - (1-S)P_D)^{R_c - a_c - d_c}$$

$$x \binom{R_T}{a_T, d_T} (St\ P_A)^{a_T} ((1-St)P_D)^{d_T} (1-St\ P_A - (1-St)P_D)^{R_T - a_T - d_T} . \quad \textbf{(1)}$$

The likelihood model is based on the following assumptions: (a) the fate of each fish is independent; (b) the control and treatment fish come from the same population of inference and share the same natural survival probability, S; (c) all alive fish have the same probability, P_A, of recapture; (d) all dead fish have the same probability, P_D, of recapture; and (e) passage survival (τ) and natural survival (S) to the recapture point are conditionally independent.

The above likelihood model has four parameters (P_a, P_D, S, τ) and four minimum sufficient statistics (a_c, d_c, a_T, d_T). The estimators associated with the above likelihood model are:

$$\hat{t} = \frac{a_T\ R_c}{R_T\ a_c}$$

$$\hat{S} = \frac{R_T\ d_c\ a_c - R_c\ d_T\ a_c}{R_c\ d_c\ a_T - R_c\ d_T\ a_c}$$

$$\hat{P}_A = \frac{d_c a_T - d_T a_c}{R_T d_c - R_c d_T}$$

$$\hat{P}_D = \frac{d_C a_T - d_T a_C}{R_c a_T - R_T a_c}.$$

The variance (Var) and standard error (SE) of the estimated passage mortality ($1-\hat{t}$) or survival ($\hat{t}$) are:

$$Var(1-\hat{t}) = Var(\hat{t}) = \frac{\hat{t}}{SP_A}\left[\frac{(1 - S\hat{t}P_A)}{R_T} + \frac{(1 - SP_A)\hat{t}}{R_c}\right],$$

$$SE(1-\hat{t}) = SE(\hat{t}) = \sqrt{Var(1-\hat{t})}.$$

The 90% confidence intervals on the estimated survival for each test condition were calculated using the profile likelihood method (Hudson 1971). The profile likelihood method constructs confidence intervals without the need to assume normality of the parameter estimates and are generally assumed superior to the normal approximations.

A likelihood ratio test was used to determine whether recapture probabilities were similar for dead (P_D) and alive (P_A) fish for each test condition (RMC and Skalski 1994a,b). The statistic tested the null hypothesis of the simplified model (H_O:P_A=P_D) versus the alternative of the most generalized model (H_A:P_A NE P_D). Depending upon the outcome of this analysis the parameters and their associated variances can be calculated using that model. The model outputs are provided in Appendix A.

Because the two test conditions (spillbays 3 and 4) were studied concurrently with a single shared control group, a modification to likelihood model (1) was used to take into account dependencies within the study design. For any two treatment groups (denoted T_1 and T_2), the modified likelihood model is as follows:

$$L(S, t, b, P_A, P_D | R_C, R_{T_1}, R_{T_2}, a_C, d_C, a_{T_1}, d_{T_1}, a_{T_2}, d_{T_2}) =$$

$$\binom{R_c}{a_c, d_c} (SP_A)^{a_c}((1-S)P_D)^{d_c} (1 - SP_A - (1-S)P_D)^{R_c - a_c - d_c}$$

$$x \binom{R_{T_1}}{a_{T_1}, d_{T_1}} (St\ P_A)^{a_{T_1}} ((1-St)P_D)^{d_{T_1}} (1 - St\ P_A - (1-St)P_D)^{R_{T_1} - a_{T_1} - d_{T_1}}$$

$$x \binom{R_{T_2}}{a_{T_2}, d_{T_2}} (S\,t^{e^b} P_A)^{a_{T_2}} ((1 - S\,t^{e^b})P_D)^{d_{T_2}} (1 - S\,t^{e^b} P_A - (1 - S\,t^{e^b})P_D)^{R_{T_2} - a_{T_2} - d_{T_2}} . \quad \textbf{(2)}$$

This likelihood has the same assumptions as model (1) and has five parameters (S, τ, ß, P_A, and P_D) that can be estimated. The survival rate for treatment T_1 is estimated by and for treatment T_2, by t^{e^b}. A likelihood ratio test with 1 degree of freedom was used to test for equality in survival rates between treatments T_1 and T_2 based on the hypothesis H_O:ß=0 versus H_a:ß NE 0. A likelihood ratio test was performed to compare recapture probabilities and reduce the dimensionability of the model and hence, improve precision if P_A=P_D. For completeness, a comparison of the parameter values estimated from each of the likelihood models is also provided in the text.

For each test condition, chi-square analyses were performed to detect homogeneity (P=0.05) within the treatment and control trials with respect to recapture rates of alive, dead, and non-recovered fish. Results of the statistical analyses, along with the derivation of variance and precision, are given in Appendix A. Summarized results are discussed in the text.